

27. (Amended) A method for preventing or treating Chlamydia infection comprising the step of administering an effective amount of the vaccine of claim 8.
28. (Amended) A method for preventing or treating Chlamydia infection comprising the step of administering an effective amount of the pharmaceutical composition of claim 10.
35. (Amended) The nucleic acid molecule according to claim 7 which is an expression plasmid selected from the group consisting of pCACPNM555a, pCAI555 and pCAD76kDa.

### **REMARKS**

Claims 1 to 38 are pending. Claims 8 to 11 and 26 to 28 are under examination.

The specification at page 11, lines 9, 11 and 12, at page 56, lines 8 and 10, and at page 60, lines 8 and 9 has been amended to insert/amend the required references to SEQ ID NOS of the Sequence Listing.

The specification at Examples 1, 2, 4, 5, 7 and 8 have been amended to specify the specific sequence cloned and expressed by SEQ ID NOS. Namely, SEQ ID NO:1 is the insert expressed from plasmid construct pCACPNM555a, SEQ ID NO:3 is the insert expressed from plasmid pCAI555, and SEQ ID NO:7 is the insert expressed from plasmid construct pCAD76kDa.

Claims 8 to 11, 26 to 28 and 35 have been amended:

Claims 8, 10 and 26 have been reformatted to independent claim format by explicitly reciting the nucleic acid of claim 1. The claims have been further amended to replace "to improve its immunogenicity" (originally in claim 1) with "without loss of immunogenicity". Support for this amendment is found at least at lines 19-23 of page 14 and lines 25-30 of page 15 of the specification.

Claim 10 has been further amended to recite that the composition comprises a carrier "or diluent suitable for use in a vaccine". The amendment is supported at least at lines 14-19 of page 10, lines 3-4 of page 28, lines 19-21 of page 31 and lines 22-34 of page 49 of the specification.

Claims 26-27 have been amended to clarify the method steps and to state use of "an effective amount". The amendment is supported at least at lines 10-17 of page 28, lines 21-26 of page 30, lines 21-27 of page 31, lines 1-5 of page 35, and lines 4-9 of page 41.

The amendment in claim 8 --wherein the vaccine vector comprises a nucleic acid molecule-- is supported at least at page 27 line 32 to page 28 line 1, page 28 lines 21 to 32, and page 29 line 6 to page 31 line 13.

The amendment in claims 8, 10 and 26 that the encoded polypeptides have been modified -- by conservative amino acid substitution-- is supported at least at page 14 lines 16 to 19 and 31 to 33, and page 15 lines 1 to 10.

The amendment in claims 8 and 10 that the nucleic acid molecule is --operatively linked to one or more control sequences for expression of the polypeptide in a mammalian cell-- is supported at least at pages 27 line 33 to page 28 line 1, page 29 lines 29 to 34, page 32 lines 1 to 4 and lines 19 to 23, as well as Examples 2, 5 and 8, where the CMV promoter is used to drive expression of the nucleic acids of the invention in mice.

The amendment in claim 8 that the nucleic acid molecule is --integrated and expressed in a bacterial cell suitable for use as a vaccine vector-- is supported at least at page 31 lines 11 to 12 where the nucleic acids are described as being inserted into the bacterial genome in bacterial vectors, and at page 24 lines 8 to 10, page 29 lines 18 to 20, and page 30 line 10 to page 15 lines 1 to 10, where bacteria suitable as vaccine vectors are described.

The amendment to claim 26 that the nucleic acid is --operatively linked to one or more control sequences for expression of the polypeptide-- is supported at least at page 24 lines 12 to 15 and page 26 line 1 to page 27 line 4.

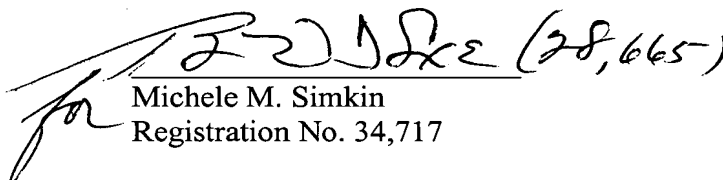
Additional amendments to claims 8 and 11 have been made to further clarify the claimed subject matter. Claim 35 has been amended to be dependent on claim 7.

No new matter has been added. Entry of the amendments is respectfully requested.

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the specification:**

Paragraph beginning at page 11, line 8, has been amended as follows:

Figure 2 shows the restriction enzyme analysis of the *C. pneumoniae* 76kDa protein gene (SEQ ID NO:1).

Paragraph beginning at page 11, line 10, has been amended as follows:

Figure 3 shows the nucleotide sequence containing a 3'-truncated 76kDa protein gene (SEQ ID NO:7) and its corresponding deduced amino acid sequence (SEQ ID NO:8) from *Chlamydia pneumoniae*; (note that nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa protein gene).

Paragraph beginning at line 25, page 51, has been amended as follows:

The full-length 76kDa protein gene (SEQ ID NO:1) was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGGTTAATCCTATTGGTCCAGG 3') (SEQ ID No:9) and a 3' primer (5' GCGCCGGATCCCTTGGAGATAACCAGAATATAGAG 3') (SEQ ID No:10). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence close to the 5' end of the full-length 76kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 76kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

Paragraph beginning at line 14, page 52, has been amended as follows:

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the full-length 76kDa protein gene (SEQ ID NO:1) was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCACPNM555a (Fig 4).

Paragraph beginning at line 5, page 56, has been amended as follows:

The 5' truncated 76kDa protein gene (SEQ ID NO:3) was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGAGTCTGGCAGATAAGCTGGG 3') (SEQ ID No:7 11) and a 3' primer (5' GCGCCGGATCCCTTGGAGATAACCAGAATATA 3') (SEQ ID No:8 12). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation

codon and a sequence at the second Met codon of the 76kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 3' 76kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

Paragraph beginning at line 27, page 56, has been amended as follows:

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the 5' truncated 76kDa protein gene (SEQ ID NO:3) was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAI555 (Fig 5).

Paragraph beginning at line 5, page 60, has been amended as follows:

The 3'-truncated 76kDa protein gene (SEQ ID NO:7 which contains SEQ ID NO:5) was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' GCTCTAGACCGCCATGACAAAAAACATTATGCTTGGG 3') (SEQ ID No:9 13) and a 3' primer (5' CGGGATCCATAGAACTTGCTGCAGCGGG 3') (SEQ ID No:10 14). The 5' primer contains a Xba I restriction site, a ribosome binding site, an initiation codon and a sequence 765bp upstream of the 5' end of the 76kDa protein coding sequence. The 3' primer includes a 21bp the sequence downstream of codon 452 of the 76kDa protein and a Bam HI restriction site. An additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag. Note that inclusion of the 765bp 5' region and the 21bp 3' regions in SEQ ID NO:7 were inadvertent. These sequences are not part of the 76kDa protein gene. Nevertheless, immunoprotection was achieved using this sequence (Example 6).

Paragraph beginning at line 30, page 60, has been amended as follows:

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Xba I/Bam HI restricted PCR fragment containing a 3'-truncated 76kDa protein gene (SEQ ID NO:7) was ligated into the Xba I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAD76kDa (Fig. 6).

**In the claims:**

Claims 8 to 11, 26 to 28 and 35 have been amended as follows:

8. (Amended) A vaccine comprising ~~at least one first nucleic acid according to claim 1, and a vaccine vector wherein each first nucleic acid is expressed as a polypeptide, the vaccine vector comprises a nucleic acid molecule which encodes a polypeptide selected from any one of:~~

(a) SEQ ID No: 2;

(b) SEQ ID No. 4;  
(c) SEQ ID No. 6;  
(d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and  
(e) a polypeptide of any one of (a) to (d) which has been modified by conservative amino acid substitution without loss of immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d); wherein the nucleic acid molecule is either operatively linked to one or more control sequences for expression of the polypeptide in a mammalian cell, or is integrated and expressed in a bacterial cell suitable for use as a vaccine vector; and  
wherein the vaccine optionally comprising comprises a second an additional nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by said first nucleic acid selected from any one of (a) to (d) above.

9. (Amended) The vaccine of claim 8 wherein the ~~second nucleic acid encodes an additional Chlamydia~~ polypeptide is a Chlamydia polypeptide.

10. (Amended) A pharmaceutical composition comprising ~~a nucleic acid according to claim 1 and a pharmaceutically acceptable carrier or diluent suitable for use in a vaccine and a nucleic acid molecule which encodes a polypeptide selected from any one of:~~

(a) SEQ ID No. 2;  
(b) SEQ ID No. 4;  
(c) SEQ ID No. 6;  
(d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and  
(e) a polypeptide of any one of (a) to (d) which has been modified by conservative amino acid substitution without loss of immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d); wherein the nucleic acid molecule is operatively linked to one or more control sequences for expression of the polypeptide in a mammalian cell.

11. (Amended) ~~A pharmaceutical composition comprising a~~ The vaccine according to claim 8 and a further comprising a pharmaceutically acceptable carrier.

26. (Amended) A method for preventing or treating Chlamydia infection ~~using the nucleic acid of claim 1 comprising the step of administering an effective amount of a nucleic acid molecule which encodes a polypeptide selected from any one of:~~

(a) SEQ ID No. 2;  
(b) SEQ ID No. 4;  
(c) SEQ ID No. 6;  
(d) an immunogenic fragment comprising at least 12 consecutive amino acids from the polypeptide of (a); and  
(e) a polypeptide of any one of (a) to (d) which has been modified by conservative amino acid substitution without loss of immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d);

wherein the nucleic acid molecule is operatively linked to one or more control sequences for expression of the polypeptide.

27. (Amended) A method for preventing or treating Chlamydia infection ~~using~~ comprising the step of administering an effective amount of the vaccine of claim 8.

28. (Amended) A method for preventing or treating Chlamydia infection ~~using~~ comprising the step of administering an effective amount of the pharmaceutical composition of claim 10.

35. (Amended) ~~An expression plasmid~~ The nucleic acid molecule according to claim 7 which is an expression plasmid selected from the group consisting of pCACPNM555a, pCAI555 and pCAD76kDa.